Prognostic significance of CD117 expression and *TP53* missense mutations in triple-negative breast cancer

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Abstract. Triple-negative breast cancer (TNBC) is extremely aggressive and associated with poor prognosis. There are no known predictive or prognostic markers for TNBC. Inhibition of tumor protein P53 (*TP53*) has been demonstrated to increase the levels of cluster of differentiation 117 (CD117) in human colorectal cancer cells. However, the function of *TP53* in the regulation of CD117 in TNBC has, to the best of our knowledge, not been reported. In the present study, the association between the expression of CD117 protein and *TP53* mutations was investigated, and their prognostic value in patients with TNBC was assessed. A total of 58 TNBC and 48 non-TNBC breast cancer tissue samples were assessed for the expression of CD117, p53 and *TP53* mutations. The marker of proliferation Ki-67 (MKI67) proliferation index and vascular invasion index (obtained by measuring D2-40 and CD34) was investigated via immunohistochemistry, and mutations in exons 4-8 of *TP53* were measured using direct sequencing. Associations between CD117 and p53 levels or *TP53* mutations and clinical parameters were statistically evaluated. The rates of CD117 or MKI67 positivity, CD117$^+$/*TP53* missense mutation$^+$, *TP53* missense mutations or recurrence were significantly higher in patients with TNBC than in patients with non-TNBC. In TNBC tissues, the presence of CD117 was associated with *TP53* missense mutations (P=0.031), vascular invasion, recurrence and MKI67. CD117$^+$/*TP53* missense mutation$^+$ also associated with vascular invasion, recurrence and MKI67. Under univariate analysis, MKI67, vascular invasion, CD117, CD117$^+$/*TP53* missense mutation$^+$ and *TP53* missense mutations were associated with the overall survival of patients with TNBC. Multivariate analysis revealed that vascular invasion and CD117$^+$/*TP53* missense mutation$^+$ in primary tumors were independent prognostic factors in patients with TNBC. In conclusion, CD117$^+$/*TP53* missense mutation$^+$ was associated with MKI67, vascular invasion and tumor recurrence in TNBC. The presence of CD117 and *TP53* missense mutations together in the primary tumors was an independent prognostic factor for survival of patients with TNBC.

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Introduction

Triple-negative breast cancer (TNBC) is characterized by the absence of estrogen, progesterone and human epidermal growth factor receptor 2 (HER2) receptors. TNBC makes up between 15 and 20% of all types of breast cancer, are aggressive, and are associated with poorer survival rates compared with non-TNBC (1,2). Although patients with TNBC respond to chemotherapy, they are more likely to suffer early relapse than patients with other breast cancer subtypes (3). Therefore, there is a requirement to identify prognostic and predictive markers in TNBC.

Cluster of differentiation 117 (CD117; encoded by *KIT*) regulates cellular differentiation, proliferation, adhesion and apoptosis (4). CD117 is involved in the development of several malignant tumor types including gastrointestinal stromal cell tumors, small-cell lung, ovarian and breast cancer (4-7). Immunohistochemical staining has revealed that CD117 protein is overexpressed in primary malignant tumors, including operable esophageal squamous cell carcinoma and vulvar melanoma, and may be a valuable prognostic marker in esophageal squamous cell carcinoma (8). However, the results of studies concerning the prognostic significance of CD117 in patients with breast cancer or TNBC are conflicting: Kashiwagi *et al* have suggested that CD117 protein is associated with poor prognosis (9), whereas others identified no significant association between CD117 and prognosis in breast cancer or TNBC (10,11).

The tumor protein P53 (*TP53*) gene encodes the tumor suppressor protein p53, and is activated by various stresses including genotoxic damage, hypoxia and heat shock (12,13).

TP53 mutations are most often located at exons 5-8, inhibiting the tumor suppressor activity of p53 (14). The association between *TP53* mutations, p53 expression and the prognosis of patients with breast cancer or TNBC is inconsistent (15-17); a group of patients with *TP53* missense mutations experienced the shortest survival and a reduction in relapse-free survival rates (15,16); however, there was no association between *TP53* mutations and clinical outcome in another study (17).

A previous study revealed that mutations to *TP53* upregulated CD117 expression in colon cancer cells and promoted the invasion of tumor cells (18). *TP53* mutations have been demonstrated to inactivate the microRNA miR-34, and to promote CD117 expression in human colorectal cancer cells (18); however, there are no reports regarding the association between *TP53* mutations and CD117 expression in TNBC.

In the present study, it was hypothesized that CD117 expression was associated with *TP53* mutations in patients with TNBC. The expression levels of CD117 and p53 were measured and *TP53* mutations were assessed in 58 TNBC tumor specimens and 48 control specimens, and their association with the clinicopathological characteristics of patients with TNBC was analyzed.

Materials and methods

Patients. The Ethics Committee for Human Studies at Shanghai Jiao Tong University (Shanghai, China) approved the present study, which was conducted in accordance with The Declaration of Helsinki. Participants were fully informed of the procedures, and written informed consent was obtained from each.

Between January 2008 and August 2012 at Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 58 TNBC tissues and 48 non-TNBC samples were collected during surgery. All patients were women, with a mean age of 55.30±9.95 years (range, 32-80 years). Patients with non-TNBC had a mean age of 55.27±8.75 years (range, 43-77 years); patients with TNBC had a mean age of 55.33±10.92 (range, 32-80 years). Stages of breast cancer were determined using Nottingham staging, recommended by the American Joint Committee on Cancer (19). A total of 39 specimens (20 TNBC and 19 non-TNBC cases) were determined to be stage I, and 67 specimens (38 TNBC and 29 non-TNBC cases) as stage II-III. All patients received modified radical mastectomy without preoperative chemotherapy or radiotherapy. Following surgery, routine neoadjuvant chemotherapy was administered.

The end of follow-up was June 2014. The 5-year survival rate for the entire cohort of 106 patients was 49%. The recurrence rate was 35.8%. During the follow-up period, recurrence occurred in 38 cases (30 patients succumbed to metastasis, and 8 patients succumbed whilst receiving chemotherapy). The median follow-up period was 50.6 months. The overall survival time of patients was calculated from the date of surgery to the date of mortality or the date of last contact.

Immunohistochemistry. Immunohistochemistry was performed on 4-μm-thick sections from 10% formalin-fixed paraffin-embedded (FFPE) tissue specimens as previously described (20). Sections were deparaffinized with 100% xylene (25˚C) and rehydrated through a graded ethanol series (100, 95 and 70% ethanol) and were subjected to microwave antigen retrieval by incubating sections at 98 to 100˚C in 0.01 M citrate buffer (pH 6.0) for 15 min. Antibodies targeting D2-40 (cat. no. sc-59347; 1:100) were purchased from Santa Clara Biotechnology, Inc. (Dallas, TX, USA). Other antibodies were purchased from Dako (Agilent Technologies, Inc.), directed against the following: Estrogen receptor (ER; cat. no. IS657; 1:80), progesterone receptor (PR; cat. no. IS068; 1:50), human epidermal growth factor receptor 2 (HER2; cat. no. 20027850; 1:200), CD117 (cat. no. 10109679; 1:200), CD34 (cat. no. GA632; 1:50), marker of proliferation Ki-67 (MKI67; cat. no. IR626; 1:200), and p53 (cat. no. IS616; 1:100), which reacts with an epitope between amino acids 19-26 and recognizes wild-type and mutant p53 protein. The sections were incubated with the primary antibodies for 1 h at 25˚C, followed by an incubation for 30 min at 37˚C with a horseradish peroxidase-conjugated secondary antibody [Dako EnVision+ System-HRP (DAB); Dako, Agilent Technologies, Inc.]. The slides were assessed in an auto immunostainer Link 48 (Agilent Technologies, Inc.; Dako) according to the manufacturer's protocols. Slides were counterstained with Mayer's hematoxylin. H&E staining of other slides from the same samples was also performed in order to assess the presence/absence of vascular invasion of tumors. Slides were stained with hematoxylin (Harris Formula, Surgipath Medical Industries, Inc., Richmond, IL, USA) at 25˚C for 1 min, rinsed in running distilled water (10 min), and then stained with an eosin solution (Surgipath Medical Industries, Inc.) for 1 min and washed with distilled water again, dehydrated with 100% ethanol (25˚C) and mounted.

All the results of immunohistohchemistry were blindly accessed by 2 independent pathologists with long-standing experience in the Department of Pathology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Nuclear staining >10% in tumor cells was considered to indicate p53- or MKI67-positivity. Nuclear staining in >1% of tumor cell nuclei was considered a positive indication of the presence of ER and PR. Immunostaining for HER2 and CD117 (11,21) was graded according to the percentage of cells: 0, none; 1+, <10% staining weak and incomplete; 2+, ≥10%, staining weak to moderate; and 3+, ≥10% strong staining. HER2 immunostaining was considered positive if graded 3+, or if gene amplification was confirmed by fluorescence *in situ* hybridization (FISH) in patients exhibiting 2+ immunostaining. Breast cancer samples with ≥10% positive cells and with scores ≥2 were considered to indicate CD117 positivity. Vascular invasion was identified by staining for CD34 (endothelial cell marker) or D2-40 (podoplanin, a membrane protein specific for lymphatic endothelium), in accordance with a previous study (22).

FISH. *HER2* status was detected using FISH. A PathVysion dual-color fluorescence probes kit (*HER2*/*CEP17*) was purchased from Vysis, Inc. (Abbott Pharmaceutical Co. Ltd., Lake Bluff, IL, USA). The assay was performed according to the manufacturer's protocol. All samples were analyzed using an Olympus BX51 fluorescence microscope (Olympus Corporation, Tokyo, Japan, original magnification, x1,000) equipped with a set of the appropriate filters (Vysis Inc.; Abbott Pharmaceutical Co. Ltd.). The hybridization results were evaluated by 2 independent pathologists in the Department

Table I. Polymerase chain reaction primers for the tumor protein P53 gene.

Exon	Forward, 5'-3'	Reverse, 5'-3'
4I	GCTCTTTTCACCCATCTACAG	GAAGGGACAGAAGATGACAG
4II	CTGCACCAGCAGCTCCTA	GAAGTCTCATGGAAGCCAG
5	TCACTTGTGCCCTGACTTTCA	TCTCCAGCCCCAGCTGCT
6	TTCCTCACTGATTGCTCTTAG	GACCCCAGTTGCAAACCAG
7	GCGCACTGGCCTCATCTTG	CACAGCAGGCCAGTGTGCA
8	AGGACCTGATTTCCTTACTGC	GAATCTGAGGCATAACTGCAC

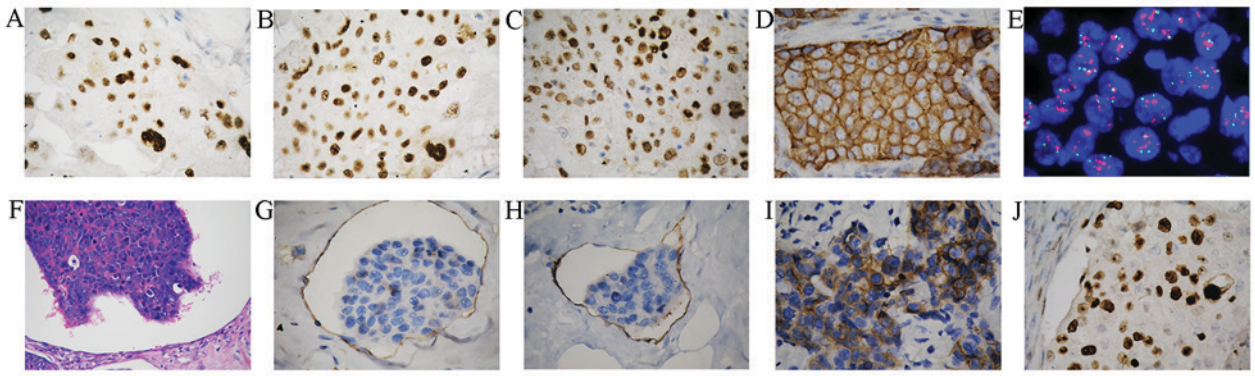

Figure 1. Immunohistochemical analysis of breast cancer tissues. Examples of positive immunohistochemical staining for the (A) estrogen receptor, (B) progesterone receptor, (C) MKI67, and (D) HER2 in tumor cells (original magnification, x400). (E) Representative image of *HER2* gene amplification in tumor tissue was observed using FISH in non-TNBC tumor cells (original magnification, x1,000). (F) Tumor thrombus was observed in vessels (original magnification, x200). (G) Blood vessel invasion was indicated by CD34 staining (original magnification, x400). (H) Lymphatic invasion indicated by D2-40 staining (original magnification, x400). Representative examples of (I) positive CD117 protein staining (J) nuclear localization of p53 and were observed in breast cancer (original magnification, x400). HER2, human epidermal growth factor receptor 2; CD, cluster of differentiation; FISH, fluorescence *in situ* hybridization; TNBC, triple negative breast cancer; MKI67, marker of proliferation Ki-67.

of Pathology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, blinded to the clinical outcomes. Categories of FISH were classified as previously described (23).

TP53 mutation analysis. For each case, 10 5-μm-thick slices were collected in a 1.5 ml tube, these FFPE specimen tissue sections were first deparaffinized with 100% xylene at 25˚C and 100% ethanol at 25˚C. Subsequently, DNA was extracted from FFPE tissue specimens using a QIAamp DNA extraction kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The samples were incubated in 20 μl proteinase K solution at 56˚C for 1 h, and then incubated in FTB buffer at 90˚C for 1 h, then wash buffers (500 μl AW1 and 500 μl AW2) washing for 1 min at 25˚C . Finally, the DNA was eluted in DTE buffer (all Qiagen GmbH).

Mutations in the *TP53* gene were identified via direct sequencing. Polymerase chain reaction (PCR) amplification and direct sequencing of the *TP53* gene (exon 4-8) were performed in 106 breast cancer samples. The primers were designed to amplify exons 4-8 of *TP53* (Table I). PCR was performed using a Mastercycler gradient PCR machine (Eppendorf, Hamburg, Germany) under the following amplification conditions: 94˚C for 10 min; 40 cycles of 94˚C for 45 sec, 61˚C for 45 sec, and 72˚C for 45 sec; with a final extension at 72˚C for 7 min. The PCR products were purified using a QIAquick Gel Extraction kit (Qiagen GmbH) and were prepared for sequencing via a 3500Dx genetic analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). The cycling conditions were: 94˚C for 1 min; 24 cycles of 94˚C for 10 sec, 50˚C for 5 sec, and 60˚C for 1 min; and final extension at 72˚C for 5 min. The sequences were analyzed using Chromas Lite software version 2.01 (Technelysium Pty Ltd., South Brisbane, Australia).

Statistical analysis. The significance of association between TNBC and the various clinical factors or potential prognostic markers was determined using the χ^2 test. Survival analysis was performed using a Kaplan-Meier plot analysis and a log-rank test. Hazard ratios with 95% confidence intervals were calculated using the Cox proportional hazards model, which was used to compute univariate and multivariate hazard ratios for the study parameters. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA).

Results

Clinical characteristics of TNBC patients. A total of 58 patients with TNBC and 48 patients with non-TNBC were included in the study. Positive stains of ER, PR, and MKI67 were primarily

Table II. Summary of clinical and pathological features of 106 patients with breast cancer.

Variable	Non-TNBC, n (%)	TNBC, n (%)	Total, n (%)	P-value
Subjects	48	58	106	
Age at operation, years				0.245
≤50	21 (43.8)	19 (32.8)	40 (37.7)	
>50	27 (56.3)	39 (67.2)	66 (62.3)	
Tumor grade				0.588
I	19 (39.6)	20 (34.5)	39 (36.8)	
II/III	29 (60.4)	38 (65.5)	67 (63.2)	
Tumor, cm				0.302
≤2	28 (58.3)	28 (48.3)	56 (52.8)	
>2	20 (41.7)	30 (51.7)	50 (47.2)	
Lymph node status				0.017
Negative	31 (64.6)	24 (41.4)	55 (51.9)	
Positive	17 (35.4)	34 (58.6)	51 (48.1)	
Vascular invasion				0.043
No	43 (89.6)	43 (74.1)	85 (80.2)	
Yes	5 (10.4)	15 (25.9)	21 (19.8)	
Nerve invasion				0.726
No	37 (77.1)	43 (74.1)	80 (75.5)	
Yes	11 (22.9)	15 (25.9)	26 (24.5)	
Recurrence				0.012
No	37 (77.1)	31 (53.4)	68 (64.2)	
Yes	11 (22.9)	27 (46.6)	38 (35.8)	

TNCB, triple negative breast cancer.

located in the nuclei (Fig. 1A-C). Immunohistochemistry revealed that HER2 protein was located at the cell membrane, and gene amplification of *HER2* was detected using FISH analysis (Fig. 1D and E). Vascular invasion was assessed by hematoxylin and eosin staining (Fig. 1F) and by immunohistochemistry using anti-CD34 (recognizing endothelial cells) and anti-D2-40 (recognizing lymphatic endothelial cells) antibodies (Fig. 1G and H). Compared with patients with non-TNBC, those with TNBC had a higher rate of vascular invasion (P=0.043), lymph node metastasis (P=0.017) and tumor recurrence (P=0.012; Table II), and a higher proliferation index (MKI67; P=0.025; Table III).

Expression of CD117 is increased in TNBC patients. CD117 expression was identified in 28/58 of TNBC samples (48.3%), and in 14/48 of non-TNBC samples (29.2%). Positive staining for CD117 was observed in the cytoplasm or at the cell membrane (Fig. 1I). The TNBC tissues exhibited a higher rate of positive staining for CD117 compared with non-TNBC tissues (P=0.045; Table III). The presence of p53 protein, localized in the nuclei (Fig. 1J), was similar in TNBC and non-TNBC patients (P=0.530; Table III). Furthermore, in TNBC, positive expression of CD117 was associated with vascular invasion (P=0.024), proliferation index (P=0.01) and tumor recurrence (P=0.001; Table IV). No association was identified between CD117 expression and tumor size, tumor grade, nerve invasion, lymph node metastasis, p53 protein or *TP53* mutation in TNBC (Table IV).

TP53 missense mutations occur at a high frequency in TNBC. Missense mutations were the most common type of *TP53* mutations (40/106, 37.7%) in breast cancer. Frame-shift mutations were identified in 7 patients (6.6%), nonsense mutations were observed in 6 patients (5.67%) and silent mutations in 3 (1.89%). A polymorphism of codon 72 in exon 4 was present in 38.68% (41/106) of patients. A codon 72 polymorphism (CCG to CCC) in exon 4 (Fig. 2A), and codon 248 mutations (CGG to TGG) in exon 7 (Fig. 2B) were the most common mutations. Wild-type codons 72, 61.3% (65/106) of breast cancer patients, and codon 248, 82.1% (87/106) of breast cancer patients were observed (Fig. 2C and D).

TP53 missense mutations in exons 4-8 were all functional mutations, with the exception of codon 72 mutations. The rate of missense mutations in TNBC tissues (33/58, 56.9%) was significantly higher than in non-TNBC tissues (17/48, 35.4%; P=0.027); however, rates of total *TP53* mutations (82.8 vs. 66.7%, respectively) and polymorphisms at codon 72 (41.4 vs. 35.4%, respectively) were similar in TNBC vs. non-TNBC groups (Table III). The presence of p53 protein was similar in the TNBC and non-TNBC patients (P=0.530; Table III). No association was identified between *TP53* mutations and the positive expression of p53 protein (Table V). *TP53* mutations

Table III. *TP53* and CD117 expression in patients with breast cancer.

Variable	Non-TNBC, n (%)	TNBC, n (%)	P-value
CD117			0.045[a]
Negative	34 (70.8)	30 (51.7)	
Positive	14 (29.2)	28 (48.3)	
CD117/*TP53*MIS			0.009[a]
Negative	42 (87.5)	38 (65.5)	
Positive	6 (12.5)	20 (34.5)	
TP53 MIS			0.027[a]
Negative	31 (64.6)	25 (43.1)	
Positive	17 (35.4)	33 (56.9)	
MKI67			0.025[a]
Negative	27 (56.3)	20 (34.5)	
Positive	21 (43.8)	38 (65.5)	
p53			0.530
Negative	31 (64.6)	34 (58.6)	
Positive	17 (35.4)	24 (41.4)	
TP53 mutation			0.055
Negative	16 (33.3)	10 (17.2)	
Positive	32 (66.7)	48 (82.8)	
Codon 72			0.530
Negative	31 (64.6)	34 (58.6)	
Positive	17 (35.4)	24 (41.4)	

[a]P<0.05. TNCB, triple negative breast cancer; CD, cluster of differentiation; *TP53*, tumor protein p53; MKI67, marker of proliferation Ki-67.

Table IV. Association of CD117 with clinicopathological features of 58 patients with TNBC.

	CD117		
Variable	Negative, n (%)	Positive, n (%)	P-value
p53			0.825
Negative	18 (60.0)	16 (57.1)	
Positive	12 (40.0)	12 (42.9)	
TP53 MIS			0.031
Negative	17 (56.7)	8 (28.6)	
Positive	13 (43.3)	20 (48.3)	
TP53 mutation			0.380
Negative	7 (23.3)	4 (14.3)	
Positive	23 (76.7)	24 (85.7)	
Codon 72			0.754
Negative	17 (56.7)	17 (60.7)	
Positive	13 (43.3)	11 (39.3)	
MKI67			0.010
Negative	15 (50.0)	5 (17.9)	
Positive	15 (50.0)	23 (82.1)	
Age, years			0.512
≤50	11 (36.7)	8 (28.6)	
>50	19 (63.3)	20 (71.4)	
Tumor grade			0.849
I	10 (33.3)	10 (35.7)	
II and III	20 (66.7)	18 (64.3)	
Tumor, cm			0.786
≤2	15 (50.0)	13 (46.4)	
>2	15 (50.0)	15 (53.6)	
Lymph node status			0.451
Negative	11 (36.7)	13 (46.4)	
Positive	19 (63.3)	15 (53.6)	
Vascular invasion			0.024
No	26 (86.7)	17 (60.7)	
Yes	4 (13.3)	11 (39.9)	
Recurrence			0.001
No	23 (76.7)	9 (32.1)	
Yes	7 (23.3)	19 (67.9)	
Nerve invasion			0.649
No	23 (76.7)	20 (71.4)	
Yes	7 (23.3)	8 (28.6)	

TNCB, triple negative breast cancer; CD, cluster of differentiation; p53/*TP53*, tumor protein p53; MKI67, marker of proliferation Ki-67.

(P=0.001) were associated with tumor recurrence, whereas expression of p53 protein was not associated with tumor recurrence (Table V). *TP53* missense mutations were associated with tumor recurrence (P=0.006), but not tumor grade, size, vascular invasion or lymph node metastasis (Table V).

Expression of CD117 is associated with TP53 missense mutations in TNBC. The TNBC tissues exhibit a higher rate of CD117 positive/*TP53* missense mutations (20/58, 34.5%) than non-TNBC tissues (6/48, 12.5%; P=0.009; Table III). The presence of CD117 was associated with *TP53* missense mutations (P=0.031) in TNBC (Table IV). The CD117 positive/*TP53* missense mutations were associated with vascular invasion (P=0.016), proliferation index (P=0.004) and particularly tumor recurrence (P<0.001). There was no association identified between CD117 positive/*TP53* missense mutations and age, grade, tumor size, node status and nerve invasion (Table VI).

CD117$^+$/TP53 missense mutation$^+$ and vascular invasion are independent prognostic factors in TNBC. Survival analysis of the 58 patients with TNBC revealed that tumor grade, vascular invasion, expression of CD117 and MKI67 protein, *TP53* missense mutations, *TP53* mutations or the CD117$^+$/*TP53* missense mutation$^+$ (CD117 and TP53 missense mutations together), were associated with overall survival (Fig. 3 and Table VII). The following features were not associated with the overall survival rate of patients with TNBC: Age, tumor size, lymph node status, nerve invasion, codon 72 mutations or expression of p53 protein. The Cox proportional hazards model revealed that CD117$^+$/*TP53* missense mutation$^+$

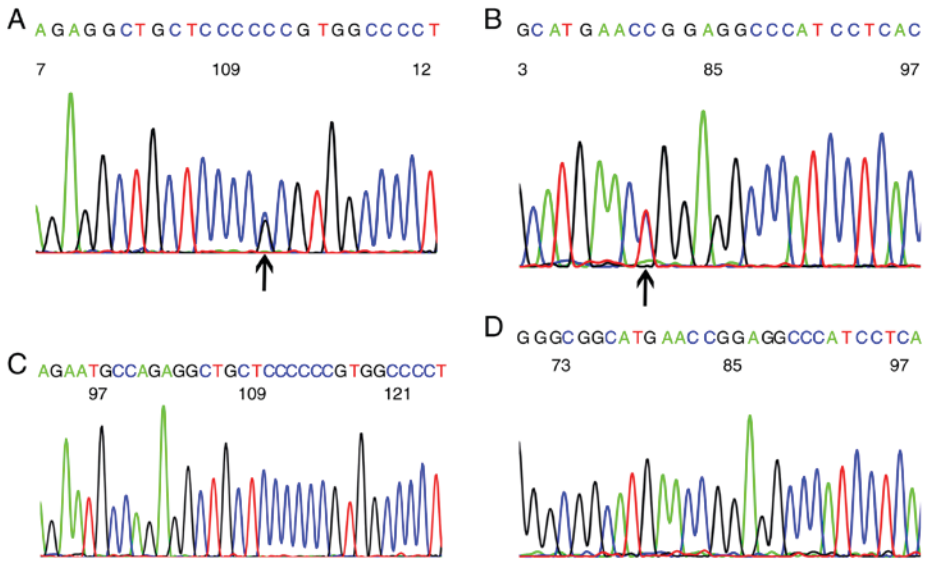

Figure 2. *TP53* gene mutations in breast cancer. (A) Codon 72 polymorphism identified in breast cancer (black arrow). (B) Codon 248 point mutations marked by black arrow were observed in breast cancer. (C) Codon 72 with no mutation. (D) Codon 248 with no mutation. *TP53*, tumor protein P53.

[positive vs. negative group, P=0.004, RR=3.153; 95% confidence interval (CI), 1.430-6.952] and vascular invasion (present vs. not present, P=0.043, RR=2.234; 95% CI, 1.026-4.863) were independent prognostic factors in patients with TNBC (Fig. 3; Table VII).

Discussion

In the present study, patients with TNBC had a higher rate of CD117 expression, *TP53* missense mutations and CD117$^+$/*TP53* missense mutation$^+$ than patients with non-TNBC. CD117, *TP53* missense mutations and CD117$^+$/*TP53* missense mutation$^+$ were associated with poor prognosis and tumor recurrence in patients with TNBC. It was confirmed that CD117$^+$/*TP53* missense mutation$^+$ was an independent prognostic factor in patients with TNBC. CD117$^+$/*TP53* missense mutation$^+$ was associated with positive expression of MKI67 and vascular invasion. It was identified that tumor grade and the proliferation index (as indicated by MKI67 levels) were poor prognostic factors, associated with a high risk of relapse in patients with TNBC, consistent with previous reports (24-26). Vascular invasion, an independent prognostic factor, was associated with tumor recurrence in patients with TNBC in the present study. The data generated in the present study were consistent with that of a previous study (27), which reported that the 5-year metastasis-free survival rate in patients with TNBC and vascular invasion was lower than in TNBC patients without vascular invasion (P=0.003) (27).

Simon *et al* (28) demonstrated that none of these tumors contained mutated *KIT*; however, in the present study a higher rate of CD117 expression was identified in patients with TNBC than in patients with non-TNBC, consistent with previous studies (11,29). It was also confirmed that the presence of CD117 was associated with the expression of MKI67 and vascular invasion in patients with TNBC, consistent with previous observations (6,29).

There are conflicting reports regarding the prognostic value of CD117 in TNBC; Several reports have demonstrated that CD117 may not influence the survival of patients with TNBC (11,30), whereas Kashiwagi *et al* have confirmed a poorer outcome for CD117-positive TNBC (9). Overexpression of CD117 protein was associated with poor prognosis and recurrence in TNBC, but was not identified as an independent prognostic factor in the present study. A possible explanation for these opposing results is differences in threshold for categorizing samples as *KIT*-positive; Thike *et al* (6) used ≥1% of tumor cells as positive criteria, whereas Kashiwagi *et al* (9) set their threshold as ≥10%. In the present study, the threshold was set at 10% of stained cells. Another explanation for these opposing results is differences in criteria of HER2 positive expression for the determination of TNBC. Nielsen *et al* (30) classified the HER2 expression according to a three-point scoring system, in which patients were divided into three groups (negative, weakly-positive and positive groups); overexpression of CD117 protein was considered not to be associated the poor prognosis of TNBC patients. However, in the present study, HER2 immunostaining was considered positive (graded 3+, or gene amplification confirmed by FISH), since it is a standard threshold for TNBC assessment.

A number of previous studies have investigated *TP53* gene mutations in TNBC (31). In the present study, the frequency of *TP53* missense mutations was increased in TNBC compared with non-TNBC tissues, consistent with previous observations (32). A positive association was not observed between the expression of p53 protein and *TP53* missense mutations in TNBC in the present study using direct sequencing, consistent with previous reports (33). However, Kim *et al* (34) reported that expression levels of p53 were also influenced by *TP53* mutation status and mRNA level of *TP53*. The authors detected *TP53* mutations using next-generation sequencing (34), while direct sequencing in our study. Taylor *et al* (35) found that results of p53 by IHC could identifying TP53 missense mutations in exons 4-8 in breast cancer, because moderate/average nuclear staining intensity for *TP53* as a positive criterion, rather than the >10% positive tumor cells that was used in the present

Table V. Association between clinicopathological features and *TP53* missense mutations.

Variable	*TP53* missense mutations		P-value
	Negative	Positive	
MKI67			0.442
Negative	10 (40.0)	10 (30.3)	
Positive	15 (60.0)	23 (69.7)	
p53			0.853
Negative	15 (60.0)	19 (57.6)	
Positive	10 (40.0)	14 (42.4)	
Age, years			0.502
≤50	7 (28.0)	12 (36.4)	
>50	18 (72.0)	21 (63.6)	
Tumor grade			0.059
I	12 (48.0)	8 (24.2)	
II and III	13 (52.0)	25 (75.8)	
Tumor, cm			0.971
≤2	12 (48.0)	16 (48.5)	
>2	13 (52.0)	17 (51.5)	
Lymph node status			0. 853
Negative	10 (40.0)	14 (42.4)	
Positive	15 (60.0)	19 (57.6)	
Vascular invasion			0.135
No	21 (84.0)	22 (66.7)	
Yes	4 (16.0)	11 (33.3)	
Recurrence			0.006
No	19 (76.0)	13 (39.4)	
Yes	6 (24.0)	20 (60.6)	
Nerve invasion			0.746
No	18 (72.0)	25 (75.8)	
Yes	7 (28.0)	8 (24.2)	

TP53/p53, tumor protein p53; MKI67, marker of proliferation Ki-67.

Table VI. Association of CD117$^+$/*TP53* missense mutation$^+$ with clinicopathological features of 58 patients with TNBC.

Variable	CD117/*TP53* missense		P-value
	Negative	Positive	
MKI67			0.004
Negative	18 (47.4)	2 (10.0)	
Positive	20 (52.6)	18 (90.0)	
Age, years			0.792
≤50	12 (31.6)	7 (35.0)	
>50	26 (68.4)	13 (65.0)	
Tumor grade			0.270
I	15 (39.5)	5 (25.0)	
II and III	23 (60.5)	15 (75.0)	
Size, cm			0.360
≤2	20 (52.6)	8 (40.0)	
>2	18 (47.4)	12 (60.0)	
Lymph node status			0.877
Negative	16 (42.1)	8 (40.0)	
Positive	22 (57.9)	12 (60.0)	
Vascular invasion			0.016
No	32 (84.2)	11 (55.0)	
Yes	6 (15.8)	9 (45.0)	
Recurrence			<0.001
No	28 (73.7)	4 (20.0)	
Yes	10 (26.3)	16 (80.0)	
Nerve invasion			0.913
No	28 (73.7)	15 (75.0)	
Yes	10 (26.3)	5 (25.0)	

TNBC, triple negative breast cancer; CD, cluster of differentiation; MKI67, marker of proliferation Ki-67.

study. Furthermore, a previous study reported the Arg72Pro polymorphism in the TNBC NIPBC-2 cell line (36). In the present study, the Arg72Pro polymorphism was identified in 41.4% of patients with TNBC and in 35.4% of the non-TNBC patients; the positive rate in patients of TNBC compared with in the non-TNBC patients was similar (P=0.530).

It has been reported that p53 expression is associated with the prognosis of patients with TNBC (15). The present study revealed that *TP53* missense mutations were associated with poor patient prognosis and a high risk of relapse in patients with TNBC, which was consistent with a previous report (37). Silwal-Pandit *et al* identified that *TP53* mutations in TNBC are not associated with an unfavorable prognosis (38). *TP53* mutations were also detected in exon2-8 (38), whereas the present study analyzed *TP53* missense mutations in exon4-8.

To the best of our knowledge, the present study reported an association between CD117 expression and *TP53* missense mutations for the first time, but not the association between CD117 expression and codon 72 polymorphisms, or synonymous mutations in patients with TNBC. CD117$^+$/*TP53* missense mutation$^+$ was associated with positive MKI67 expression, vascular invasion and tumor recurrence. On the basis of these results, it may be hypothesized that CD117$^+$/*TP53* missense mutation$^+$ is associated with the malignant biological behavior of patients with TNBC. p53 has been reported to regulate numerous genes, including MYC proto-oncogene and *KIT*, affecting the aggressive biological behavior of cancer (39-41). p53 may also directly regulate microRNA-34 (miR-34), with inactivation of p53 reducing miR-34 expression, promoting the invasion of colon cancer cells (18). Furthermore, the present study demonstrated that the expression of CD117 protein in TNBC tissues was an independent prognostic factor in patients with TNBC with *TP53* missense mutations.

It was not possible to perform sub-categorization of patients with TNBC according to biological characteristics such as EGFR amplification, which may be one limitation of the present study. CD117$^+$/*TP53* missense mutation$^+$ was

Table VII. Univariate and multivariate survival analysis of 58 patients with TNBC.

Variable	P-value		RR	95% CI
	Univariate	Multivariate		
Age, years (≤50 vs. >50)	0.738			
Tumor size, cm (≤2 vs. >2)	0.389			
Lymph node status (+ vs. -)	0.601			
Histological grade (high vs. low)	0.014			
Vascular invasion (yes vs. no)	<0.001[a]	0.043[a]	2.234	1.026-4.863
Nerve invasion (yes vs. no)	0.898			
CD117 (+ vs. -)	<0.001			
MKI67 (+ vs. -)	0.040			
P53 (+ vs. -)	0.651			
TP53 MIS (+ vs. -)	0.011			
TP53 mutation (+ vs. -)	0.022			
Codon 72 (+ vs. -)	0.837			
CD117/TP53MIS (+ vs. -)	<0.001[a]	0.004[a]	3.153	1.430-6.952

[a]Statistically significant. CI, confidence interval; RR, risk ratio; CD, cluster of differentiation; MKI67, marker of proliferation Ki67; *TP53*, tumor protein P53.

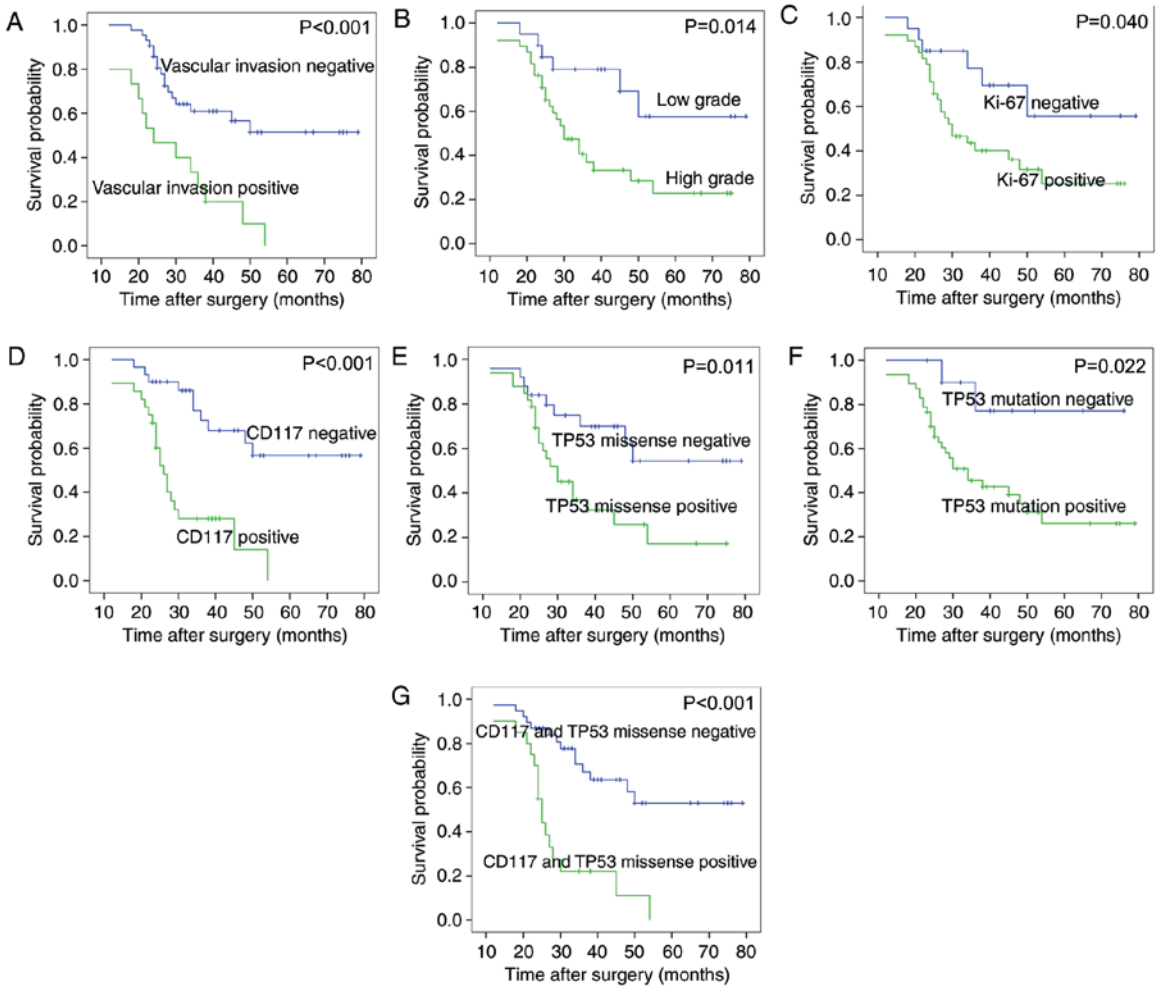

Figure 3. Survival analysis in patients with TNBC. Kaplan-Meier plots of overall survival in patients with TNBC and with (A) positive and negative vascular invasion, (B) high and low grade tumors, (C) positive and negative MKI67 protein expression, (D) CD117 protein expression, (E) *TP53* missense mutations, (F) *TP53* mutations, and (G) CD117 with *TP53* missense mutations. TNBC, triple negative breast cancer; MKI67, marker of proliferation Ki-67; TP53, tumor protein P53.

not analyzed in patients with TNBC of different genotypes. The present study also did not detect the level of miR-34 or analyze the association between miR-34 and CD117$^+$/*TP53* missense mutation$^+$. Therefore, further study into the effect of CD117$^+$/*TP53* missense mutation$^+$ in patients with TNBC with different genotypes and the association between miR-34 and CD117$^+$/*TP53* missense mutation$^+$ is required.

In conclusion, the present study confirmed that CD117$^+$/*TP53* missense mutation$^+$ was associated with MKI67 expression, vascular invasion and tumor recurrence in patients with TNBC. CD117 expression was indicative of poor prognosis in patients with TNBC with *TP53* missense mutations.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author, on reasonable request.

Authors' contributions

Preparation of the DNA, sequencing, IHC and analysis of data were performed by YLL. Statistical analysis was also performed by YLL. The slides were analyzed by HZZ and WTH. GL participated in analysis of experimental data and conducted the PCR experiment. The manuscript was written by YLL and reviewed by HZZ and GL .

Ethics approval and consent to participate

The Ethics Committee for Human Studies at Shanghai Jiao Tong University (Shanghai, China) approved the present study, which was conducted in accordance with The Declaration of Helsinki. Participants were fully informed of the procedures, and written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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